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H. K. Abbas[a]; B. J. Johnson[a]; D. J. Pantone[b]; R. Hines[c]
[a] USDA-ARS SWSRU MS 38776 Stoneville. [b] TAES Texas A&M University TX 76502 Temple. [c] University of Illinois Department of Crop Sciences, DSAC IL 62985 Simpson.

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SHORT COMMUNICATION

Biological Control and Use of Adjuvants against Multiple Seeded Cocklebur (*Xanthium strumarium*) in Comparison with Several Other Cocklebur Types

H. K. ABBAS[1], B. J. JOHNSON[1], D. J. PANTONE[2] AND R. HINES[3]

[1]*USDA-ARS, SWSRU, Stoneville, MS 38776, USA;* [2]*TAES, Texas A&M University, Temple, TX 76502, USA;* [3]*University of Illinois, Department of Crop Sciences, DSAC, Simpson, IL 62985, USA*

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Common cocklebur has several biotypes including multiple seeded cocklebur (MSC), NCC-TX, and NCC-MS. Alternaria helianthi *applied at* 2.5×10^4 *conidia* mL^{-1} *in a 50% micro-emulsion of unrefined corn oil (MESUCO) or 0.2% Silwet L 77 caused 60–75% mortality on NCC-TX and MSC. Increasing the conidial concentration to* 5×10^4 mL^{-1} *increased mortality to 100% on MSC and NCC-TX, and 75% on NCC-MS. At* 10×10^4 *conidia* mL^{-1}, A. helianthi *caused 100% mortality in all three biotypes. No mortality occurred in any biotype at inoculation rates of 2.5 and* 5×10^4 *conidia* mL^{-1} *when applied in water. Increasing the dew period from 0 to 12 h increased mortality from 0 to 100% on all three biotypes at a rate of* 2.5×10^4 *conidia* mL^{-1} *in Silwet and MESUCO. MSC appears to be the most sensitive biotype.*

Keywords: *Cocklebur (*Xanthium strumarium *L.) biotype, multiple seeded cocklebur, biological control,* Alternaria helianthi*, formulation*

Common cocklebur (*Xanthium strumarium* L.) is an economically significant annual weed in many crops including cotton, corn, and soybean, causing yield reduction (Buchanan & Burns, 1971; Barrentine, 1974; Holm *et al*., 1977). Common cocklebur has many biotypes including those resistant to conventional herbicides such as the monosodium salt of methylarsenic acid (MSMA) (Haigler *et al*., 1988; Barrentine, 1994). Also, this genus contains biotypes that vary in their growth and development (Zimmerman & Weis, 1983; Tranel & Wassom, 2001). A unique biotype, called multiple-seeded cocklebur (MSC), was

Correspondence to: Dr. Hamed K. Abbas, Lead Scientist, Crop Genetics & Production Research Unit, 141 Experiment Station Road, P.O. Box 345, Stoneville, MS 38776, USA. Tel: +1-662-686-5313; Fax: +1-662-686-5218; E-mail: habbas@ars.usda.gov

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discovered in Bell County, TX, USA, in 1994. This biotype has up to 25 seeds/bur, usually producing up to nine seedlings. MSC burs are large, round, and flattened on one end (Abbas *et al*., 1999).

Alternaria species are important pathogens of a wide range of weed and crop species (Rotem, 1994; Abbas & Barrentine, 1995). Allen *et al*. (1983) suggested that common cocklebur, which is also a member of the Asteraceae family, may serve as another host in addition to sunflower of *A. helianthi* (Hansf.) Tubaki and Nishihara. Quimby (1989) substantiated this observation. *A. helianthi* has now been well documented as an effective pathogen to control common cocklebur (Abbas & Barrentine, 1995; Abbas *et al*., 1995; Abbas & Egley, 1996), but it has never been tested against MSC.

Some research has demonstrated that Silwet (SW) promotes the activity of bacteria and fungi on the leaves of their host kudzu (*Pueraria* sp.) (Zidack *et al*., 1992; Boyette *et al*., 2002). Also, corn oil has been shown to enhance the efficacy of several mycoherbicides such as *A. helianthi* (Abbas & Egley, 1996), *Colletotrichum truncatum* (Boyette, 1994; Egley & Boyette, 1995) and *C. orbiculare* (Klein *et al*., 1995). Therefore, in the present study, the response of MSC and normal common cocklebur (NCC) varieties to the potential mycoherbicide agent *A. helianthi* in various geographical areas was compared. Another aim of the study was to determine if common cocklebur can be controlled with very low rates of fungal conidia.

Seeds of MSC and normal common cocklebur-Texas (NCC-TX) were collected from plants growing in experimental plots at the Southern Weed Science Research Nursery, Stoneville, MS. The original sources of seeds of both biotypes are described in Abbas *et al*. (1999). Normal common cocklebur from Mississippi (NCC-MS) was collected from local areas at Stoneville, MS. All burs except MSC burs were cut with a saw between the two beaks, lengthwise, while attempting to avoid any damage to the seeds as described in Abbas *et al*. (1999). MSC burs were not cut because seeds were not symmetrical and no difficulty was encountered with germination. The process was repeated until enough seeds were obtained for these studies.

Methods of Abbas *et al*. (1995) were employed for growth, preparation and application of *A. helianthi* inoculum as were the greenhouse conditions used for producing cocklebur plants and examining their response to infection by *A. helianthi*. The effects of different inoculum rates of *A. helianthi* conidia were assessed on all biotypes of cocklebur. The plants were prepared as described by Abbas and Barrentine (1995) and Abbas *et al*. (1999).

Eight- to 12-leaf growth stages (GS) of MSC-TX and six- to eight-leaf GS of NCC are comparable because of the larger number of leaves associated with MSC-TX. Briefly, MSC usually develops sequential sets of four true leaves in whorls. MSC produces two cotyledons, four true leaves, eight true leaves, and 10 or 12 true leaves at the same time that NCC has two cotyledons, then one set of two true leaves, and then another set of two true leaves. These two true leaves are in alternate positions and so on. The development of NCC is as follows: two cotyledons; two, four, and six true leaves and so on. Therefore, the six- to eight-leaf GS in NCC is equivalent or exactly the same as the eight- to 12-leaf GS in MSC, and usually MSC leaves are smaller (Abbas *et al*., 1999).

The eight- to 12-leaf GS of MSC-TX and the six- to eight-leaf GS of other cocklebur were inoculated with four rates of inoculum: 0, 2.5, 5 and 10×10^4, conidia mL^{-1} of distilled water or two different adjuvants. The first adjuvant was an organo-silicone surfactant, polyalkyleneoxide modified heptamethyltrisiloxane (Silwet L77; Union Carbide, Tarrytown, NY, USA). The second adjuvant was a micro-emulsion of unrefined corn oil (MESUCO) (Spectrum Natural Inc., Petaluma, CA, USA). To prepare this adjuvant, a 50% (v/v) unrefined corn oil and distilled water mixture was homogenized for 3–5 min at high speed using a Brinkmann Polytron® Homogenizer, PT 3000 (Brinkmann Instruments Inc., Westbury, NY, USA) at 27.3×10^3 rpm. The homogenate was poured into a 2-L separation funnel. After 30–60 min, the white to light yellow coloured lower fraction was collected, and

the dark, thick yellow or golden upper fraction was discarded. This light yellow fraction was easy to spray and was not phytotoxic. Conidia in water, 0.2% SW, or MESUCO were sprayed with an atomizer ('Spra-Tool'; Crown Industrial Products Co., Hebron, IL, USA) until run-off, requiring 3 mL/plant on 14–21-day-old common cocklebur seedling (six- to 12-leaf stage). Four replicates with 10 plants/treatment were used. Treated and control plants were subjected to an 8-h dew period prior to transferring them to the greenhouse for 14 days. The plants were watered, fertilized with (N:P:K, 20:20:20) as needed and greenhouse temperature was maintained between 28 and 32°C with 40–60% RH. The photoperiod was *ca.* 14 h at *ca.* 1600–1800 μE m^{-2} s^{-1} photosynthetic photon flux density at midday. Mortality was measured at the end of the experiment (14 days). The experiment was repeated twice and results were pooled.

Plants were grown and inoculated as described before with 2.5×10^4 conidia mL^{-1} suspended in distilled water formulated in two adjuvants. Treated and control plants were subjected to dew periods of 0, 4, 6, 8, 12, 16, 20 or 24 h prior to transferring them to the greenhouse where they were maintained for 14 days. Plants were watered as needed and fertilized with (N:P:K, 20:20:20). Plant mortality was measured at the end of the experiment (14 days). The experiment was repeated twice and results were pooled.

Statistical analysis of cocklebur mortality data (Tables 1 and 2) was carried out by analysis of variance (ANOVA) to demonstrate significant differences in mortality with different formulations ($P < 0.001$, both studies), biotypes ($P < 0.01$, both studies) and inoculum sizes ($P < 0.001$, Table 1) or dew periods ($P < 0.001$). Tukey's honest significant difference (HSD) method was used at the 95% confidence level to determine the significance of differences between individual formulations, biotypes, inoculum sizes and dew periods.

A. helianthi caused severe growth reduction of treated plants. The injury started as soft necrotic lesions on leaf surfaces and stems, which became more severe and larger with time, covering the whole plant and resulting in mortality within 1 week. When formulated at 2.5 and 5×10^4 conidia mL^{-1} in distilled water, *A. helianthi* did not cause mortality to six- to 12-leaf GS of the three cocklebur biotypes tested, when sprayed to run-off with 8-h dew period (Table 1).

TABLE 1. Effect of inoculum rate of *A. helianthi* on percentage mortality of three cocklebur types

		% Mortality at inoculum rates (conidia mL^{-1})			
Biotype[a]	Formulation	0	2.5×10^4[c]	5×10^4[c]	10×10^4
MSC-TX	H_2O	0[b]	0	0	10
NCC-TX	H_2O	0	0	0	5
NCC-MS[d]	H_2O	0	0	0	0
MSC-TX	Silwet[e]	0	75	100	100
NCC-TX	Silwet	0	60	100	100
NCC-MS[d]	Silwet	0	15	75	100
MSC-TX	50% MESUCO[e]	0	75	100	100
NCC-TX	50% MESUCO	0	70	100	100
NCC-MS[d]	50% MESUCO	0	20	75	100

[a]Cocklebur biotypes used: MSC-TX, multiple seeded cocklebur-Texas; NCC-TX, normal common cocklebur-Texas; and NCC-MS, normal common cocklebur-Mississippi.

[b]Values are means of two separate experiments, each consisting of four replicates containing 10 plants at the six- to 12-leaf GS/replicate (one plant/pot).

[c]Significantly greater mortality than with the lower inoculum rates tested ($P < 0.05$, Tukey's HSD).

[d]Significantly more susceptible to *A. helianthi* than biotypes from Texas (NCC-TX, MSC-TX) ($P < 0.05$, Tukey's HSD).

[e]Significantly higher mortality than with the H_2O formulation ($P < 0.05$, Tukey's HSD).

TABLE 2. Mortality (%) of cocklebur biotypes – response to various dew periods when treated with *A. helianthi* at a rate of 2.5×10^4 conidia mL^{-1} under greenhouse conditions

		Dew period (h)					
Biotype[a]	Formulation[b]	0	4	6[d]	8[d]	12[d]	24[d]
MSC-TX	H_2O	0[c]	0	0	0	10	100
NCC-TX	H_2O	0	0	0	0	0	75
NCC-MS[e]	H_2O	0	0	0	0	0	75
MSC-TX	Silwet[f]	0	0	50	80	100	100
NCC-TX	Silwet	0	0	30	75	100	100
NCC-MS[e]	Silwet	0	0	20	40	100	100
MSC-TX	50% MES[f]	0	0	50	80	100	100
NCC-TX	50% MES	0	0	40	80	100	100
NCC-MS[e]	50% MES	0	0	30	50	100	100

[a]Three cocklebur biotypes were used: NCC-MS, normal common cocklebur-Mississippi collected locally; NCC-TX, normal common cocklebur-Texas; MSC-TX, multiple seeded cocklebur-Texas.

[b]A rate of 2.5×10^4 conidia mL^{-1} distilled water (H_2O), 0.2% Silwet, and 50% micro-emulsion solution (MES) of unrefined corn oil were used to treat plants.

[c]Values are the means of two separate experiments, each consisting of four replicates containing 10 plants at the six- to 12-leaf GS/replicate (one plant/pot).

[d]Significantly greater mortality than with the shorter dew periods tested ($P < 0.05$, Tukey's HSD).

[e]Significantly more susceptible to *A. helianthi* than biotypes from Texas (NCC-TX, MSC-TX) ($P < 0.05$, Tukey's HSD).

[f]Significantly higher mortality than with the H_2O formulation ($P < 0.05$, Tukey's HSD).

A. helianthi was significantly ($P < 0.05$, Tukey's HSD) more active against cocklebur plants at the six- to 12-leaf GS when formulated in SW or MESUCO than when formulated with water, but there was no significant difference in mortality between the two adjuvants in either the study on the effect of inoculum size (Table 1) or on the effect of dew period (Table 2). Among the biotypes examined in the two studies (Tables 1 and 2), NCC-MS was significantly ($P < 0.05$, Tukey's HSD) less susceptible to *A. helianthi* than the two biotypes from Texas, and there was no significant difference in susceptibility between NCC- and MSC-TX from Texas. Increasing inoculum rate (Table 1) resulted in significantly ($P < 0.05$, Tukey's HSD) increased mortality with increasing inoculum rate up to 10×10^4 conidia mL^{-1}, at which no significant increase in mortality occurred. After an initial 4-h lag period, mortality increased significantly ($P < 0.05$, Tukey's HSD) with increasing dew period up to 24 h. However, the use of either adjuvant (SW or MESUCO) decreased the length of dew period required for extensive mortality from 24 h to more practical levels such as 8 h.

This will make biological control of common cocklebur more feasible and agrees with previous studies (Zidack *et al*., 1992; Boyette, 1994; Egley & Boyette, 1995; Abbas & Egley, 1996; Quimby *et al*., 2003). The precise mode by which the corn oil emulsion and the surface-active agent enhance conidial germination is yet to be determined. It was found in this study that 0.2% SW formulation enhances fungal efficacy and reduces the fungal dew period required for the infection process. It may be that SW causes fungal conidia to adhere more closely to the leaf tissues than fungal conidia in water or oil formulations. Silwet may stimulate spore germination of *A. helianthi*, allowing the conidia to produce multiple germ-tubes to penetrate the stomata or possibly wounds caused by SW as well. Previous results have shown that conidia germinated more rapidly when in close contact with glass, filter paper and leaf tissue surface (Abbas *et al*., unpublished data). Germination of fungal conidia of the mycoherbicide *C. truncatum* on a solid surface such as glass microscope slides and detached plant leaves are well documented by Egley and Boyette (1995). Also, unrefined corn oil has also been shown to enhance the efficacy of several mycoherbicides such as *A. helianthi* (Abbas & Egley, 1996) and *Colletotrichum truncatum* (Egley & Boyette, 1995). It has been reported that oil in a water emulsion formulation enhances mycoherbicide efficacy by

providing a water supply required by the fungus. Also, the formulation may enhance conidial penetration into leaf tissue (Greaves *et al.*, 2001).

MSC has characteristics that suggest that it might be difficult to control biologically or chemically or both, particularly the fact that it produces several seedlings/bur, giving the possibility of faster spread. However, the results of the current study demonstrate that MSC is susceptible to biological control by *A. helianthi*. Therefore, more research is needed to find ways to enhance the activity of this agent against various biotypes of cocklebur. Virulent conidia in copious amounts are needed. As discovered by Quimby (1989), it is difficult to produce sufficient conidia of this fungus for it to be economically feasible. The rationale of this study was to test if this biological agent could control cocklebur at very low rates. This study met this goal but not without dew and the use of some adjuvants. It is possible that with advanced techniques the goal of producing copious amounts of conidia by this fungus might be achieved.

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